Claims

1. A method of making a substantially pure culture of ES cell-derived keratinocytes comprising:

expanding a keratinocyte cell harvested from an ES cell nodule to obtain a substantially pure culture of ES cell-derived keratinocytes.

2. The method of claim 1, wherein the cells harvested from the ES cell nodule are contacted with low Ca^{++} medium to selectively deplete ES cells from the harvested cells.

3. The method of claim 1, wherein the embryonic stem (ES) cell nodule is a human ES cell nodule.

4. The method of claim 1, wherein the embryonic stem (ES) cell nodule is prepared in a *scid* mouse.

5. The method of claim 1, wherein the means of harvesting the keratinocyte cell from the ES cell nodule comprises disaggregation of the ES cell nodule.

6. The method of claim 5, wherein the disaggregation of the ES cell nodule comprises contacting the ES cell nodule with trypsin.

7. The method of claim 1, wherein the harvested keratinocyte cell is expanded in low Ca^{++} medium with or without 3T3 cells or other strain of embryonic fibroblast.

8. The method of claim 7, wherein the low Ca^{++} medium is serum-free medium.

9. The method of claim 1, wherein the harvested keratinocyte cell is expanded in cFAD medium with or without 3T3 cells or other strain of embryonic fibroblast.

10. The method of claim 9, wherein the cFAD medium comprises 10% (v/v) fetal calf serum.

11. The method of claim 1, wherein the keratinocyte cell is first expanded for one or more passages in low-Ca^{++} medium with or without 3T3 cells or other strain of embryonic fibroblasts and subsequently expanded for one or more passages in cFAD medium with or without 3T3 cells or other strain of embryonic fibroblast.

12. The method of claim 11, wherein the low Ca^{++} medium is serum-free medium.

13. The method of claim 11, wherein the cFAD medium comprises 10% (v/v) fetal calf serum.

14. The method of claim 1, wherein the cells of keratinocyte lineage are cells that display one or more markers selected from the group consisting of: p63, K14, basonuclin, involucrin, colony fragmentation and circumferential movement.

15. A product formed by the method of any one of claims 1-14.

16. The method of claim 1, further comprising administering keratinocytes from the substantially pure culture of ES cell-derived keratinocytes to a subject for the treatment of a wound.

17. The method of claim 1, further comprising administering a composition comprising keratinocytes from the substantially pure culture of ES cell-derived keratinocytes to a subject for the treatment of a wound.

18. A method of making a substantially pure culture of embryonic stem (ES) cell-derived keratinocytes comprising:

expanding selectively a keratinocyte derived from cultured embryonic stem (ES) cells to obtain a substantially pure culture of ES cell-derived keratinocytes.

19. The method of claim 18, wherein the embryonic stem cells are an aggregate.

20. The method of claim 19, wherein the aggregate comprises two or more human embryonic stem cells.

21. The method of claim 19, wherein the aggregate is a human embryoid body or a mega-embryoid body.

22. The method of claim 21, wherein the aggregate is cultured on a surface adapted for cell attachment, for a time sufficient to permit cells to grow and migrate distally from the aggregate.

23. The method of claim 22, wherein the cells that migrate distally away from the cultured aggregate are cells of keratinocyte lineage.

24. The method of claim 22, wherein the surface adapted for cell attachment is a cell culture dish.

25. The method of claim 22, wherein the time sufficient to permit cells to grow and migrate distally from the human embryoid body is at least about 10 days.

26. The method of claim 25, wherein the cells are permitted to grow and migrate distally from the human embryoid body for about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 days.

27. The method of claim 22, wherein the time sufficient to permit cells to grow and migrate distally from the mega-EB is at least about 1 day.

28. The method of claim 27, wherein the cells are permitted to grow and migrate distally from the mega-EB for at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 days.

29. The method of claim 19, wherein the aggregates are cultured in cFAD medium on irradiated 3T3 cells or other strain of embryonic fibroblast.

30. The method of claim 18, wherein the cells of keratinocyte lineage are expanded in serum-free medium with or without irradiated 3T3 cells or other strain of embryonic fibroblast.

31. The method of claim 18, wherein the cells of keratinocyte lineage are first expanded for one or more passages in low-Ca^{++} serum-free medium with or without 3T3 cells or other strain of embryonic fibroblast and subsequently expanded for one or more passages in cFAD medium with or without 3T3 cells or other strain of embryonic fibroblast.

32. The method of claim 31, wherein the cFAD medium comprises 10% (v/v) fetal calf serum.

33. The method of claim 18, wherein the cells of keratinocyte lineage are cells that display one or more markers selected from the group consisting of: p63, K14, basonuclin, involucrin, colony fragmentation, and circumferential movement.

34. A product formed by the method of any one of claims 18-33.

35. The method of claim 18, further comprising administering keratinocytes from the substantially pure culture of ES cell-derived keratinocytes to a subject for the treatment of a wound.

36. The method of claim 18, further comprising administering a composition comprising keratinocytes from the substantially pure culture of ES cell-derived keratinocytes to a subject for the treatment of a wound.

37. A method of treating a skin injury in a subject comprising:

administering to a subject in need of such treatment an ES cell-derived keratinocyte made with the method of any of claims 1-14 or 18-33 in an amount effective to treat the skin injury.

38. The method of claim 37, wherein the skin injury is the result of disease or trauma.

39. The method of claim 38, wherein the trauma is a burn.

40. A method of identifying an ES cell-derived cell for treating an injury in a subject comprising,

contacting an ES cell-derived cell in culture with retinoic acid,

determining the presence of circumferential movement in the contacted cell, wherein the presence of circumferential movement identifies the cell for treating injury in the subject.

41. The method of claim 40, wherein the retinoic acid is at a concentration in the culture of between about 10^{-7} molar and about 10^{-10} molar.

42. The method of claim 40, wherein the ES cell-derived cell is an ES cell-derived keratinocyte.

43. The method of claim 40, wherein the ES cell-derived keratinocyte is an ES cell-derived keratinocyte made with the method of any of claims 1-14 or 18-33.

44. A method of identifying an ES cell-derived keratinocyte for treating an injury in a subject comprising,

culturing an ES cell-derived cell, wherein the cell forms a colony,

determining the presence of fragmentation of the colony, wherein the presence of the fragmentation identifies the keratinocyte for treating injury in the subject.

45. The method of claim 44, wherein the ES cell-derived cell is a cell from an ES cell nodule.

46. The method of claim 44, wherein the ES cell-derived keratinocyte is an ES cell-derived keratinocyte made with the method of any of claims 1-14 or 18-33.

47. A composition comprising a embryonic stem cell-derived keratinocyte made with the method of any of claims 1-14 or 18-33, or identified with the method of any of claims 40-43 or 44-46.

48. A method of treating a skin injury in a subject comprising:

obtaining an ES cell-derived keratinocyte made with the method of any of claims 1-14 or 18-33 and

administering the ES cell-derived keratinocyte to a subject in need of such treatment in an amount effective to treat the skin injury.

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49. The method of claim 48, wherein the skin injury is the result of disease or trauma.

50. The method of claim 49, wherein the trauma is a burn.